Lab: 5 Biochemical tests used for identification of medical bacteria

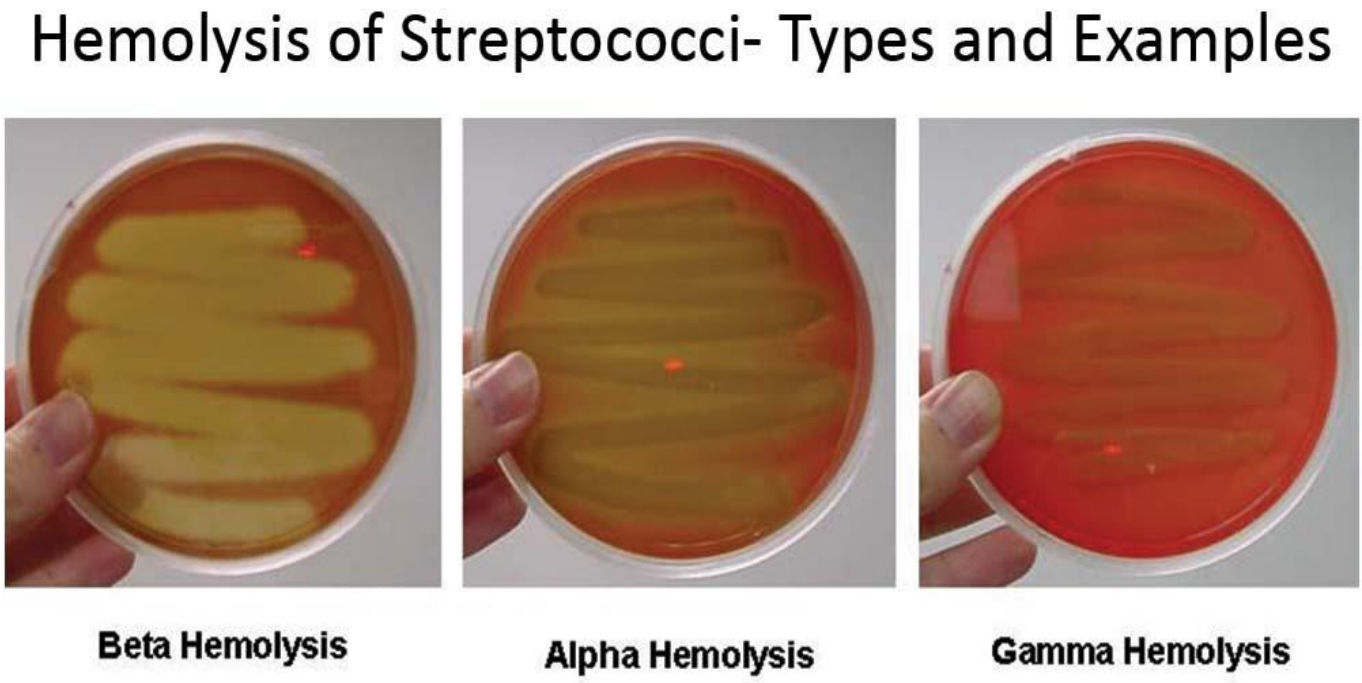
Biochemical tests have an important role in the identification of bacteria to classify bacteria and determine the causative agent of diseases.

1-Haemolysis: Some types of pathogenic bacteria are able of producing haemolysin enzyme that lyses Erythrocytes (RBCS). This can be detected in vitro on blood agar plates. There are three types of haemolysis:

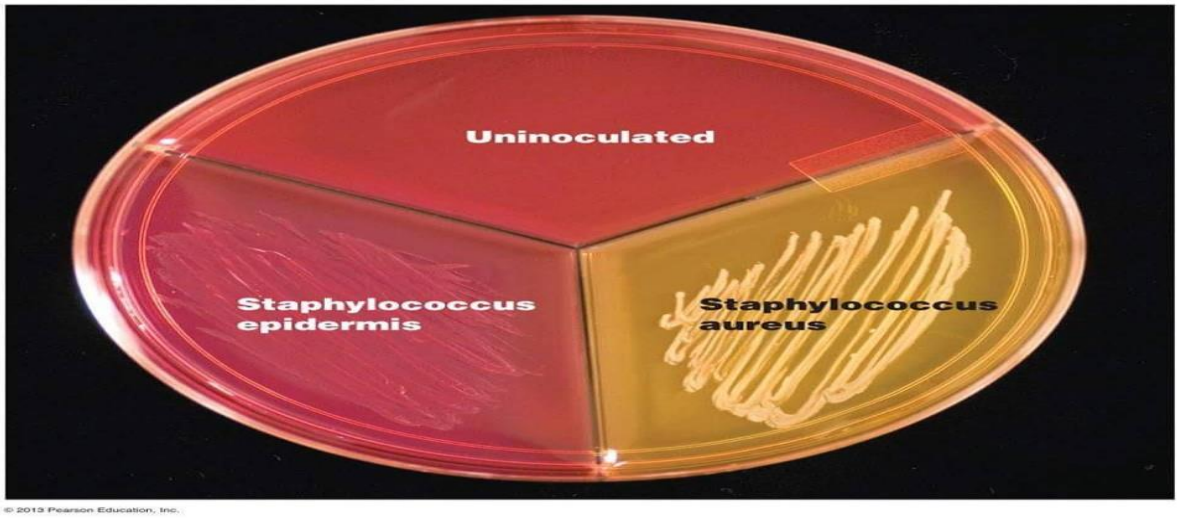
A- β-haemolysis: Complete clear circular zone around the bacterial colonies due to complete lysis of red cells. e.g. Streptococcus pyogenes and Staphylococcus aureus

B- α-haemolysis: appear as greenish zone around the colonies· due to partial haemolysis of RBCs. e.g. Streptococcus viridians

C- γ-haemolysis: (no haemolysis) no any obvious changes around the colonies e.g. Enterococcus Faecalis



2- **Mannitol fermentation:** This can be detected in vitro on mannitol salt agar plates. Staphylococcus aureus can be ferment the sugar (mannitol) in this media &become yellow, while S. epidermidis cannot ferment the sugar &become white.



3-**Pigment production:** Some type of bacteria able to produce a characteristic pigments. There are two types of pigments:

Endopigment: Remain bound to the body of the M.O. and doesn't diffuse to the surrounding media e.g. Serratia and Staphylococcus

Exopigment: Soluble which readily diffuse into the surrounding media e.g.Pseudomonas aerogenosa produce four types of pigments Pyocyanin (blue-gree) Pyoveridin (green), Pyorubin (red) and Pyomelanin (black)

4- Motility test: Motility of bacteria can be detected by several methods; used to determine whether an organism is equipped with flagella or not e.g:-

1- Hanging drop technique

2-Stabbing of semisolid medium- .

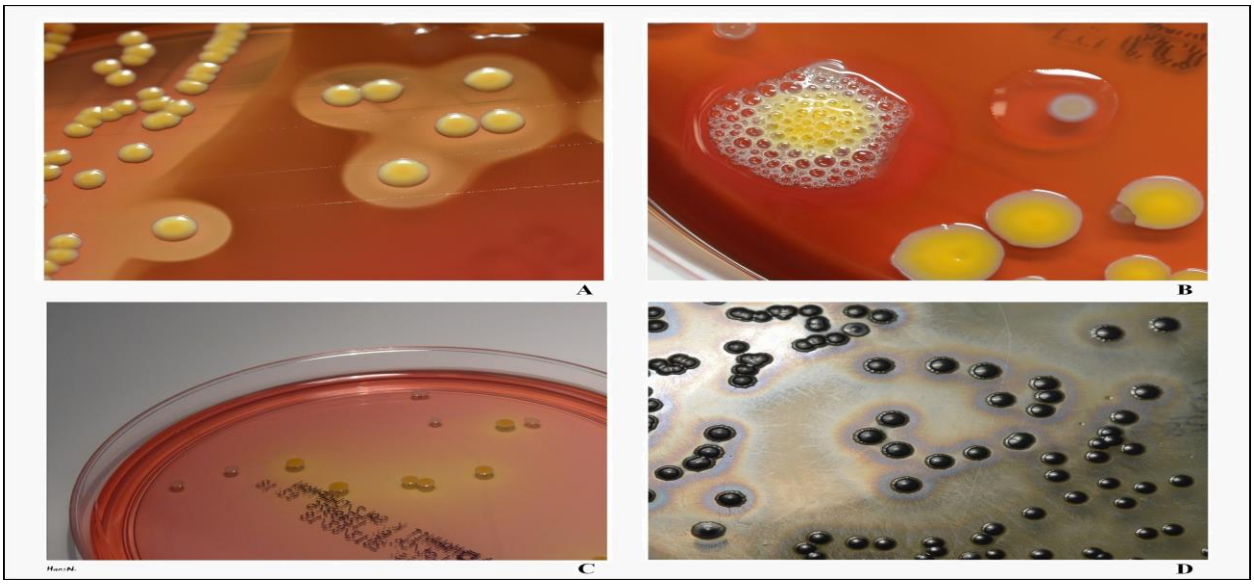
3-Flagellar stain

Motile bacteria such as Salmonella, Proteus and E coli

5-Catalase production test: Some aerobic bacteria able to produce catalase enzyme that catalyses H2O; (Hydrogen peroxide) and releases O2 and H2O

$2O2 + 2H$ — catalase —> $O2 + 2H2O$

Procedure: A small amount of bacterial culture to be tested is picked from nutrient agar by stick or glass rod and put it on the surface of a clean slide, where a drop of (3 %H2O) was added. Formation of gas bubbles indicates a positive result. A false positive reaction may obtain if the culture medium contain catalase (Blood agar) or if iron loop is used.



6-Coagulase production: Some bacteria produce coagulase enzyme that converts soluble fibrinogen protein to insoluble fibrin protein (coagulation of plasma).Coagulase is a virulence factor of Staphylococcus aureus. The formation of clot around an infection caused by this bacterium will protects it from phagocytosis

A-Bound coagulase (Slide method)

B- Free coagulase (Tube method)

7-Oxidase test: Use to detect the production of cytochrome oxidase which related to respiratory electron transport chain and it produced by strictly aerobic bacteria e.g. Pseudomonas and Neisseriae.

Procedure: A small area of filter paper is soaked with a freshly prepared 1% oxidase reagent (Tetramethyl-p-pheuylene Diamine Dihydrochloride) bacterial colony to be tested is picked from agar by stick or glass rod and put it on the soaked area. A positive result is indicated by formation of deep purple color due to reduction of this dye by oxidase enzyme.

8-Triple sugar iron (TSI) and Kligler's iron agar (KIA)

TSI medium contain (glucose, lactose and sucrose)

KIA contain only (glucose and lactose)

*pH indicator: phenol red (red in alkaline pH and yellow in acidic pH).

*Ferrous sulfate as an indicator of H2S production

These media are used to detect ability of bacteria to ferment these sugars and this aid in the identification and classification of enteric G-ve bacilli)enterobacteriaceae).

Three criteria can be detected:

l- Bacterial ability to produce gas from sugar fermentation. This makes the media to push up or break up.

2-H2S gas production can be detected by the production of black precipitate in the bottom of the media. As H2S react with iron in the media to form black ferrous sulfide in the butt.

3-Ability to ferment sugars that can be detected by color changes from red to yellow. Position of the color change distinguishes the acid production associated with glucose fermentation from the acidic products of lactose or sucrose fermentation. Bacteria that ferment glucose produce acid that turn the color of the pH indicator to yellow in the butt but not in the slant (result——> K/A). While lactose or sucrose fomenters produce more acid that turn both butt and slant to yellow (result—> A/A)

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Lab5:- Biochemical tests used for identification of medical bacteria

9-Urease test: This test is used to identify bacteria able of hydrolyzing urea using the enzyme urease to make ammonia and carbon dioxide. The hydrolysis of urea raises the pH to above 7.0 and the pH indicator (phenol red) turns the medium from yellow to red pink.

$NH_2\text{-}CO\text{-}NH_2 + H_2O$ — urease —>$2NH_3 + CO_2$

Urea ammonia

Ex: of urease producer are Helicobacter pylori and V. cholera , Klebsiella & Proteus

10-IMVC: These are a group of biochemical test that help in the identification and differentiation between enteric G-ve bacilli (enterobacteriaceae).

A-Indole production test: It tests for the bacterial ability to produce indole. Bacteria use an enzyme, tryptophanase to break down the amino acid (tryptophan) to give indole, ammonia and pyruvic acid.

Tryptophan — Tryptophanase —> Indole + ammonia + pyruvic acid

Peptone liquid medium containing tryptophan is inoculated the- tested bacteria and incubated at 37 °C for 24 hrs. Few drops of kovac's reagent are added to the bacterial growth. The presence of red rig in the superficial layer of the medium indicate +ve result of indole production e.g. E.coli. Yellow ring indicate —ve result e.g. Klebsiella.

B- Methyl red/ Voges-Proskauer tests: Both MR and VP tests are used to determine what end products result when the tested organism degrades glucose (for energy production) and this depend on the type of enzyme that the bacteria have